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Iron Absorption by Iron-Efficient and -Inefficient Species of Apples

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ABSTRACT

Four apple species, *Malus xiaojinensis* Cheng et Jiang, *M. micromalus* Makino, *M. transitoria* (Batal.) Schneid, and *M. baccata* (L.) Borkh., were investigated for their differences in iron (Fe) uptake mechanisms. The results showed that *M. xiaojinensis* and *M. micromalus* had higher root CEC than *M. transitoria* and *M. baccata*. There seems to be an 'Fe pool' in the root apparent free space (AFS) of these species, being highest in *M. baccata*. Iron content in the root free space of *M. xiaojinensis* and *M. micromalus* changed with higher external Fe level (40 μ M) and/or higher pH (7.8), whereas that of *M. baccata* and *M. transitoria* was not significantly changed. *Malus xiaojinensis* and *M. micromalus* had higher electrical conductivity than *M. baccata* and *M. transitoria*. Under Fe-stress condition, *M. xiaojinensis* decreased the rhizosphere pH as much as 2 units as compared to about 1 unit with *M. micromalus*. With regard to Fe absorption rate, *M. xiaojinensis* showed two absorption peaks, one at the higher Fe concentration of above 64 μ M, which was the same as with the other test species, and another one at 2 μ M Fe which was not observed in the other species. The Fe absorption rate of *M. micromalus*

increased with increasing levels of external Fe concentration. The results obtained support our conclusion in a previous paper that of the four species used in the experiments, *M. xiaojinensis* was the most Fe efficient. *Malus micromalus*, next to *M. xiaojinensis*, was moderately Fe efficient, as evidenced by its high CEC, high root free space Fe content, high electrical conductivity, its ability to lower the rhizosphere pH under high pH + low Fe condition, and a strong ability to absorb Fe from the nutrient solution.

INTRODUCTION

Concentration of soluble Fe is very low in soil solution, and Fe is moved to the root surface mainly via diffusion, whereas the diffusion coefficient for Fe in soils is very small. Therefore, Fe absorbed by crops came mostly from the rhizosphere (Brown et al., 1971). Some physiological changes in Fe-efficient dicotyledons could be induced under Fe-deficiency stress, including increased exudation of H^+ (Chaney et al., 1972; Devos et al., 1986; Olsen and Brown, 1980; Römheld et al., 1984; Venkat-Raju et al., 1972), reducing substances like phenolics (Brown and Ambler, 1973; Marschner et al., 1974; Römheld and Marschner, 1981), and lowering rhizosphere pH (Chaney et al., 1989; De Vos et al., 1986; Korcak, 1987; Römheld et al., 1982; Römheld et al., 1984).

An Fe pool existed in the root AFS of the tested crops, and Fe concentration could reach 500-1000 μM g⁻¹ FW in the Fe Pool for soybean and maize when FeEDTA was used as an Fe source (Bienfait et al., 1985). Longnecker and Welch (1988) reported that Fe-efficient soybean cultivar, HA, accumulated a large portion of Fe in the root AFS to form an Fe pool. The Fe in the pool could be activated and transported to above-ground parts of crops under Fe-deficiency stress. In their further study, Longnecker and Welch (1990) certified the above-stated results, and pointed out that the genetic variances among different resistant crops to Fe deficiency were related to amount of Fe accumulated in root AFS and utilization of the Fe under Fe-deficiency condition.

Summarizing other workers' reports, Liu (1988) found a linear relationship between cation exchange capacity (CEC) of root and cation contents in the above-ground parts of crops. The higher the plant root CEC was, the more plants were known to absorb cations. A correlation was also noticed between Fe and manganese (Mn) contents and root CEC, and Liu believed that root CEC could be used as a reliable parameter for screening Fe- or Mn-efficient cultivars.

In our previous report, it was concluded that of the four apple species under test, *Malus xiaojinensis* Cheng et Jiang appeared to be the most Fe efficient, whereas the other three species, *M. micromalus* Makino, *M. transitoria* (Batal.) Schneid, and *M. baccata* (L.) Borkh., were Fe inefficient, exhibiting varying degrees of inefficiency (Han et al., 1994). This paper discusses follow-up experiments conducted to compare the differences in some aspects of the mechanisms of Fe uptake of these four species.

MATERIALS AND METHODS

Root Characteristics

Thirty-five day-old seedlings of four apple species grown in perlite were transferred to a nutrient solution as reported in our previous experiment (Han et al., 1994). The treatment consisted of (1) pH6.0+LFe, (2) pH6.0+HFe, (3) pH7.8+LFe, and (4) pH7.8+HFe; where LFe and HFe denotes 10 μ M and 40 μ M Fe respectively. After one week of growth, plants were removed for the determination of the parameters below.

Root Cation Exchange Capacity

Roots were washed 3 times in distilled water, oven dried at 80°C, and ground, and 0.5 to 1.0 gram of ground sample was soaked in 200 mL 0.01N HCl for 5 min, filtered, and washed several times with distilled water. The precipitate was soaked in 200 mL pH7.0 1M KCl, and after measuring the pH, titrated with 0.01N KOH to pH7.0.

Root Apparent Free Space Fe Content

One to 3 g of distilled water washed roots were mixed with 4°C distilled water at 1:20 ratio of root:water. The mixture was stirred and stored at 4°C for 2 hr and then filtered. The Fe in the infiltrate was determined by ICP.

Electrical Conductivity

Seedling roots were washed in distilled water and immersed in the above-stated solution for 1 hr. Electrical conductivity of the solution was measured using DDS-11A Electrical Conductivity Analyzer (Shanghai Second Analyzer Instrument Company, Shanghai, China).

Rhizosphere pH

Fifty day-old seedlings, grown in perlite, were grown for an additional 3-week period in nutrient solution similar to the earlier section. After the growth period, the seedlings were removed and the roots were washed in distilled water, and embedded in dyed agar medium for 0.5 to 3 hr. The dyed agar medium was prepared by mixing 1 g of agar, 13.6 mg CaSO_4 , and 6 mg Bromocresol purple into 100 mL distilled water, and heated. After cooling the medium to 40~50°C, the pH was adjusted to 6.0. The rhizosphere pH was measured by a pH meter, using a slightly modified method of Römheld et al. (1981).

Iron Absorption Rate

After growing for 45 days in the nutrient solution as previously conducted (Han et al., 1994), the seedlings were transferred into deionized distilled water.

TABLE 1. Root cation exchange capacity of the four tested species at different levels of pH and Fe (micro equivalent/100 g DW).

Species	pH6.0+LFe	pH6.0+HFe	pH7.8+LFe	pH7.8+HFe
<i>M. xiaojinensis</i>	672.72 ^{A_D}	1157.36 ^{A_A}	756.46 ^{A_C}	850.89 ^{A_B}
<i>M. micromalus</i>	641.62 ^{B_C}	1053.11 ^{A_A}	550.09 ^{B_C}	756.06 ^{A_B}
<i>M. transitoria</i>	420.31 ^{C_B}	539.41 ^{B_A}	374.34 ^{C_B}	410.16 ^{B_B}
<i>M. baccata</i>	302.05 ^{D_A}	655.92 ^{B_A}	267.85 ^{D_A}	494.97 ^{B_A}

Note: Values followed by the same letter do not differ at the 0.01% probability. Letters at the upper right corner denote comparisons among species and at the lower right corner denote comparisons among treatments within the same species.

Twelve hours later, they were transferred into Fe-treatment solutions containing 0, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048, and 4096 μM Fe, respectively, using FeEDTA as the Fe source. The pH value of the treatment solutions was adjusted to 7.8. The experiments were carried out according to the following procedures.

Excised Root

The seedlings were separated into root and shoot. The root was cut to pieces of 1-3 cm in length, and root surface water was absorbed using tissue paper. Half a (0.5) g root per treatment was placed into 500 mL of the aerated Fe-treatment solution with a pH of 7.8. It was shaken at 25°C, and 25 oscillation/min for 6 hr, after which the root pieces were taken out of the solution, and rinsed with deionized distilled water for 1 min. The samples were placed into paper bags and oven dried at 70°C for 3 days. The Fe content of the samples was analyzed by ICP.

Intact Root

These samples were immersed in the Fe-treatment solutions. After shaking at 25°C and 25 oscillation/min for 6 hr, the intact root was separated from the seedlings, rinsed immediately with deionized distilled water, oven dried at 70°C for 3 days, and ground for analysis of Fe by ICP.

RESULTS

Root Cation Exchange Capacity

At both solution pHs and Fe treatments, *M. xiaojinensis* and *M. micromalus* exhibited significantly higher root CEC than the other two species (Table 1). The

TABLE 2. Iron content in the root apparent free space of the tested species (mg/L).

Species	pH6.0+LFe	pH6.0+HFe	pH7.8+LFe	pH7.8+HFe
<i>M. xiaojinensis</i>	13.75 ^b _b	17.36 ^c _a	14.91 ^c _{ab}	16.33 ^c _{ab}
<i>M. micromalus</i>	18.06 ^b _b	23.19 ^b _a	21.01 ^b _{ab}	23.84 ^b _a
<i>M. transitoria</i>	12.52 ^b _a	14.06 ^c _a	12.44 ^c _a	9.89 ^d _a
<i>M. baccata</i>	34.68 ^a _a	36.41 ^a _a	32.03 ^a _a	30.40 ^a _a

Note: Values followed by the same letter do not differ at the 0.01% (capital letter) or 0.05% (small letter) probability. Letters at the upper right corner denote comparisons among species and at the lower right corner denote comparisons among treatments within the same species.

differences among species were especially evident at the low Fe treatment. At the high Fe treatment and at both pH levels, *M. baccata* gave significantly higher CEC than *M. transitoria*, which indicated that, at adequate supply of Fe, the former has benefited from higher an Fe uptake than the latter.

At both low and high Fe levels, higher CEC was noted for three of the *Malus* species at pH6.0 than pH7.8, with the exception of *M. xiaojinensis*. At low Fe treatment, *M. xiaojinensis* had a higher CEC at pH7.8 than at pH6.0, indicating that this species was more tolerant of sustaining low Fe in the growth medium at higher pH, and supported the conclusion of our previous report (Han et al., 1994).

Iron Content in Root Apparent Free Space

Under nutrient stress, ions that were present in root AFS were readily available to the plant (Marschner et al., 1974). Regardless of Fe or pH levels, *M. baccata* was the highest in Fe content in root AFS (Table 2). For the other three species, significant differences in Fe content in root AFS was only observed in the treatment with pH7.8+HFe. Among the three species, *M. micromalus* had the highest Fe concentration in root AFS, which was followed by *M. xiaojinensis* and *M. transitoria*.

The Fe content of *M. xiaojinensis* at pH6.0+LFe was significantly lower than that at the other treatments, and so was *M. micromalus*. With *M. transitoria* or *M. baccata*, their Fe content in root AFS at different pH or Fe levels were not significantly different.

Electrical Conductivity

Plants were known to secrete higher amounts of exudates under nutrient stress, which tend to increase nutrient availability (Liu, 1988). Increasing amounts of

TABLE 3. Electrical conductivity of the tested species (μ /g FW 400 mL).

Species	pH6.0+LFe	pH6.0+HFe	pH7.8+LFe	pH7.8+HFe
<i>M. xiaojinensis</i>	1.825 ^{A_{ab}}	0.959 ^c	2.576 ^{A_a}	1.495 ^{A_b}
<i>M. micromalus</i>	0.841 ^{b_{ab}}	0.510 ^{ab_b}	1.084 ^{b_a}	0.734 ^{b_{ab}}
<i>M. transitoria</i>	0.303 ^{b_a}	0.284 ^{b_a}	0.322 ^{c_a}	0.298 ^{b_a}
<i>M. baccata</i>	0.369 ^{b_a}	0.326 ^{b_a}	0.402 ^{c_a}	0.350 ^{b_a}

Note: Values followed by the same letter do not differ at the 0.01 (capital letter) or 0.05 (small letter) percent probability. Letters at the upper right corner denote comparisons among species and at the lower right corner denote comparisons among treatments within the same species.

exudates tended to enhance the electrical conductivity of the medium (Römheld et al., 1982). Electrical conductivity of *M. xiaojinensis* was the highest among the four species regardless of pH or Fe treatments (Table 3). With the exception of treatment pH6.0+LFe, *M. micromalus* exhibited significantly lower electrical conductivity than *M. xiaojinensis* and significantly higher electrical conductivity than the other two species. However, the electrical conductivity of the solution, at both pH and Fe treatments, were similar in *M. micromalus* and *M. baccata*. Electrical conductivity in *M. xiaojinensis* and *M. micromalus* was higher at low Fe and both pH levels. The two *Malus* species yielded higher values with pH7.8 irrespective of the Fe levels. Electrical conductivity of the other two species were not influenced by treatment effects.

Rhizosphere pH

High pH and low soil Fe were unfavorable for the absorption of Fe by plants (Korcak, 1987). The four species used in this study reduced their rhizosphere pH at high pH and both levels of Fe (Table 4). The decrease of pH in the rhizosphere of *M. xiaojinensis* at pH7.8 with 10 μ M Fe amounted 1.41 pH units. However, only a slight change of rhizosphere pH occurred with all the four species at low pH and high Fe, a condition favorable for Fe absorption. In general, pH levels of the culture solution appeared to exert a stronger influence in lowering the rhizosphere pH than Fe level.

Iron Absorption Rate

Absorption of Fe by intact and excised roots increased with increasing concentration of Fe in the medium (Figure 1). An absorption maximum was noticed around 128 μ M in *M. xiaojinensis* and *M. baccata*, and at 64 -128 μ M in

TABLE 4. Rhizosphere pH of the tested species.

Species	pH6.0+LFe	pH6.0+HFe	pH7.8+LFe	pH7.8+HFe
<i>M. xiaojinensis</i>	6.18	6.91	5.37	6.26
<i>M. micromalus</i>	6.92	7.13	6.39	7.01
<i>M. transitoria</i>	6.77	7.19	6.87	6.77
<i>M. baccata</i>	6.5	6.72	6.38	6.62

M. transitoria. A minor peak of absorption was observed at 2-4 μM in *M. xiaojinensis* and at 512-1024 μM in *M. baccata*. The Fe absorption in *M. micromalus* remained low until Fe concentration of the culture medium reached 60 μM , after which a rapid rate of Fe absorption was observed. Maximum absorption of 120 mg L^{-1} in *M. xiaojinensis* and 400 mg L^{-1} in *M. micromalus* were noticed at Fe concentration of 2048 μM . This was lower in *M. transitoria* and *M. baccata*, where rates of 100 and 80 mg L^{-1} , respectively, were noticed. In all the species, excised root consistently absorbed less Fe than intact roots. Similar observations have been reported for barley by Bloom and Caldwell (1988).

DISCUSSION

The results indicated that root CEC controls selective absorption of ions in the root Donnan free space, which influences the components of mineral elements absorbed by the root. The higher the root CEC is, the higher the absorption of cations by the four *Malus* species was. *M. xiaojinensis* and *M. micromalus*, and especially the former, showed much higher CEC than the two other species, indicating that their absorption capacity for Fe was rather strong. Our results also showed that Fe concentration, and not pH of the nutrient solution, was the main factor affecting root CEC.

Electrical conductivity of *M. xiaojinensis* was the highest among the four species used in this experiment. This, together with the pronounced decrease of the rhizosphere pH, pointed to the fact that the roots of *M. xiaojinensis* secreted a greater amount of ions and other substances into the culture solution which facilitated Fe absorption. Just what ions and other substances were secreted remained to be determined. Brown and Draper (1980) stated that Fe-efficient blueberries were capable of lowering solution pH by proton release from the roots, but Fe-inefficient plants did not change the solution pH. The blueberries did not release reductants, and Fe-efficient blueberry crosses all contained less calcium (Ca) than the Fe-inefficient crosses. Olsen and Brown (1980), working with

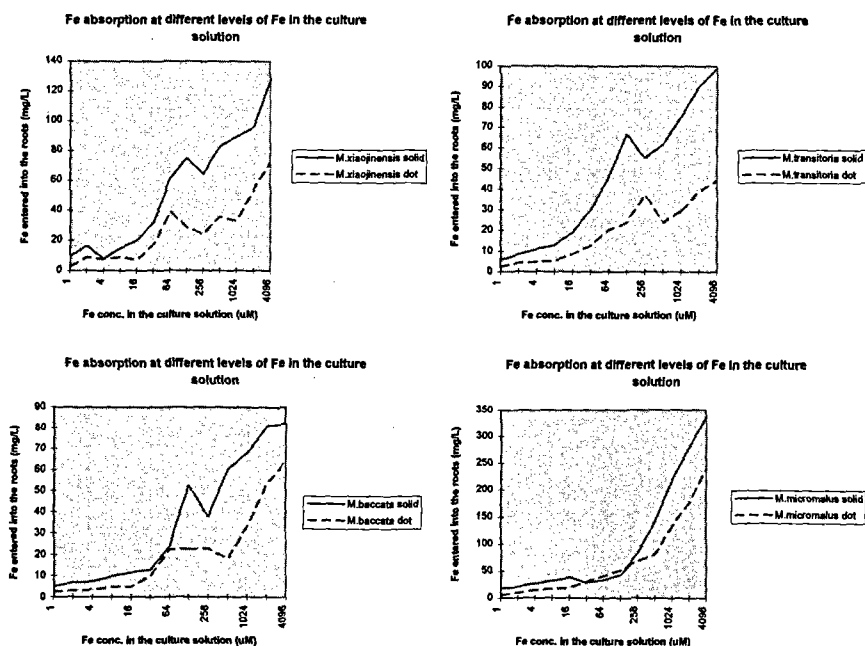


FIGURE 1. Iron absorption of the four tested species at different levels of iron in the culture solution. Solid lines=intact apple root; dotted lines=excised apple root.

tomato, maize, soybean, and oats, concluded that the Fe-efficient genotype was the only species of these four to lower solution pH via H^+ efflux from the roots significantly and released reductants. The Fe-efficient soybeans released reductants but did not lower solution pH. It was known that the capacity of roots to reduce rhizosphere pH and/or release reductants into the rhizosphere were two mechanisms whereby plants increased Fe uptake. Apparently, there was considerable variation in the capacity to enhance Fe absorption via rhizosphere pH modification. According to Römheld et al. (1982, 1984), Sun et al. (1987), Korcak (1987), and Chaney et al. (1989), rhizosphere pH was lowered under Fe-stressed condition. In our experiments, however, this was true only with *M. xiaojinensis* and *M. micromalus*, both being Fe-efficient species, especially the former.

It is to be noted that *M. baccata*, a species known to induce serious Fe-deficiency chlorosis on calcareous soils, had the highest Fe content in the root free space of the four species under test, forming an Fe pool. It would be expected that upon impending Fe deficiency, the plant could mobilize Fe from this free space Fe pool. However, *M. baccata* apparently was unable to accomplish such mobilization. Bienfait et al. (1985), working with bean, chlorophytum and maize,

found that maize was not able to mobilize either its free space Fe pool upon Fe deficiency.

Malus xiaojinensis had two Fe-absorption peaks, the first one occurred at low and medium Fe treatment (2 or 4 μM). This was perhaps due to the fact that reacting to Fe-stressed condition, this species mobilized Fe from the Fe pool in the root free space. Part of the soluble Fe in the Fe pool (Korcak, 1987) was directly absorbed by Fe-stressed roots of plant (Leigh and Wyn, 1973). The other part of Fe in the Fe pool was insoluble Fe, which was reduced to ferrous Fe by reductants secreted by roots under Fe-deficiency stress (Chaney et al., 1972). The high root CEC and its capacity of lowering the rhizosphere pH enhanced Fe absorption by *M. xiaojinensis* even at low Fe level in the culture solution.

Malus micromalus differed from the other three species in the absence of an absorption peak midway between the low and high levels of Fe in the nutrient solution. Furthermore, the amount of Fe entering into the roots was much greater than in the three other species at the same Fe concentration of Fe in the culture solution. This could imply that *M. micromalus* had a strong ability to absorb Fe from the medium by some mechanism not yet known. This, together with its high CEC, high root free space Fe content, high electrical conductivity, and its ability to markedly lower the rhizosphere pH under high pH + low Fe condition, would provide evidence to consider *M. micromalus* as an Fe-efficient species, next to *M. xiaojinensis*.

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